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RESEARCH ARTICLE

SYNTHESIS OF FLAVONE ANALOGUES OF [1,2,4]TRIAZOLO[3,4-*b*][1,3,4]THIADIAZOLE AS ANTIBACTERIAL AGENTS

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ABSTRACT

A new series of 2-phenyl-3-(6-aryl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-4*H*-4-chromenone 10(a-j) has been synthesized from 3-(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)-2-phenyl-4*H*-4-chromenone 9. All newly synthesized compounds were screened for their *in vitro* antibacterial activities against bacterial strains of *Bacillus subtilis*, *Micrococcus luteus*, *Proteus mirabilis*, *and Escherichia coli*. The activity of compounds containing 2-chloropheny (10b), 4-chlorophenyl (10c) and 4-methoxyphenyl (10f) are nearly equal to that of standard drug. The compound with 4-hydroxyphenyl (10e) showed significant activity against *B. subtilis* and *M. luteus*. Compound 10a and 10g did not show any antibacterial activity against *P. mirabilis* and *E. coli* at test concentration.

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INTRODUCTION

The 1,2,4-triazole is an important class of heterocycle and present as core part in many biologically and pharmacologically active molecules, its *N*-bridged heterocyclic analogues have been reported as anti-inflammatory (Palaska, 2002), antidepressant (John, 1988), antiviral (Todoulou, 1994), analgesic (Shantaram, 2013) and antitumor (Romeo, 2010). In addition, it has been reported that thiadiazoles also exhibit a broad spectrum of biological effectiveness such as antiparkinsonism (Fuchs, 1988), hypoglycaemic (James, 1989), anticancer (Georgios, 2019), anti-inflammatory (Maddila, 2016), antiasthmatic (Rajive, 1988), antihypertensive (Vio, 1989), antiviral (Lu, 2017), anthelmentic (Prakash, 2008) and antituberculer (Gundurao, 2006). Further the triazole ring fused with other heterocyclic rings is also found to possess diverse applications in the field of medicine (Yao, 2005). The fused 1,2,4-triazole with 1,3,4-thiadiazole ring exhibited antimicrobial (Demirbas, 2005), antiviral (Kritsanida, 2002), anti-HIV (Invidiata, 1996), anti-inflammatory (Maddila, 2013) and herbicidal (Hiroshi, 1970) activities. Similarly, Flavones and its derivatives occupy a special place in the realm of natural and synthetic organic chemistry owing to their useful biological activities such as antioxidant (Chan, 2003), anxiolytic (De Almeida, 2009), anticancer (Liu, 1992), analgesic (Dao, 2004) and antimicrobial (Sohel, 2006). Encouraged by the biological profile, we designed a set of hybrid molecules with two different pharmacophore such as 1,2,4-triazole, 1,3,4-thiadiazole and then linked to flavones ring with the aim of increasing their antibacterial activity. We report herein the synthesis of new series of 2-phenyl-3(6-aryl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-4*H*-4-chromenone 10(a-j) and evaluation of their in vitro antibacterial activities.

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MATARIALS AND METHODS

All reagents are commercial grade and were used as supplied. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel F254 plates from Merck, and compounds visualized by exposure to UV light. Chromatographic columns 70–230 mesh silica gel for separations were used. IR spectra were recorded using KBr disk on a Perkin–Elmer FTIR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C). Chemical shifts are reported in δ ppm units with respect to TMS as internal standard and coupling constants (*J*) are reported in Hz units. Mass spectra were recorded on a VG micro mass 7070H spectrometer.

Synthesis of 4-hydroxy-2*H*-2-chromenone (2): A mixture of phenol **1** (0.01 mol), malonic acid (0.01 mol), $POCl_3$ (40 mL) and anhy. $ZnCl_2$ (30 g) were heated on a water bath at 70 ^oC for 12 h. The reaction mass was cooled and poured into ice. The solid separated was digested in 10% Na_2CO_3 and filtered. The filtrate upon acidification gave compound **2** as light yellow powder in 56% of yield, mp. 211-213 ^oC.

Synthesis of 1-[(*E*)-2-nitro-1-ethenyl]benzene (5): The benzaldehyde **3** (0.01 mol), nitro- methane **4** (0.02 mol) were added to the stirred solution of acetic acid (10 mL) and ammonium acetate (2 g). The resulting solution was refluxed in an oil bath at 110 ^oC for 6-8 h. After the clearance of TLC, the total dark solution was distilled under *vacume* completely brown colored syrup obtained, the syrup was poured in crushed ice and product was extracted with ethyl acetate (50 mL × 3), the combined organic layer dried over Na_2SO_4. The combined organic layers were evaporated under vacuum to afford the impure reaction mixture which was purified by column chromatography (silica gel) to obtain the pure β-nitrostyrene **5** as yellow solid in 67% of yield, mp. 58-60 ^oC.

Synthesis of ethyl 4-oxo-2-phenyl-4*H*-3-chromenecarboxy late (6): To a stirred solution of compound **2** (0.01 mol) and corresponding compound **5** (0.02 mol) in ethanol (6 mL) was added 4-dimethylaminopyridine (DMAP, 0.04 mol). The reaction mixture was heated at 70-80 ^oC, and the progress of the reaction was monitored by TLC of ethyl acetate and hexane (15:85%). After the formation of the product, the crude reaction mixture was extracted with EtOAc (2x10 mL), the combined organic layers were washed with H_2O (10 mL) and dried (Na_2SO_4). The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (60-120 mesh) to afford the pure products **6**. IR (KBr) ν_{max}: 3392 (O-H), 3067 (CH-Ar), 1734 (C=O), 1687 (C=O), 1652 (C=C), 1278 (C-O) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 1.32 (t, 3H, CH_3), 4.56 (q, 2H, CH_2), 7.45-7.55 (m, 6H, ArH), 7.92 (d, J = 7.9 Hz, 2H, ArH), 8.22 (d, J = 8.1 Hz, 1H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 15.6, 62.8, 110.9, 119.0, 123.2, 124.9, 125.7, 127.3, 128.4, 129.1, 132.5, 133.3, 156.6, 166.1, 170.8, 174.4; MS: *m/z* 295 (M^+ +1).

Synthesis of 4-oxo-2-phenyl-4*H*-3-chromenecarbohydr-azide (7): A mixture of compound **6** (0.01 mol) and hydrazine hydrate (0.01 mol) in ethanol (15 mL) was heated under reflux temperature for 4 hours. After completion of the reaction it was cooled and the solid separated was filtered and washed with water and then purified by recrystalization from ethanol to afford pure compound **7**. IR (KBr) ν_{max}: 3412 (N-H), 3089 (CH-Ar), 1712 (C=O), 1682 (C=O), 1649 (C=C), 1369 (C-N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 5.52 (s, 2H, NH_2), 7.45-7.55 (m, 4H, ArH), 7.70-7.80 (m, 4H, ArH), 8.21 (d, J = 8.6 Hz, 1H, ArH), 8.92 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 110.4, 119.0, 123.2, 125.1, 126.1, 127.0, 127.5, 128.9, 133.1, 135.7, 153.2, 166.8, 173.8; MS: *m/z* 281 (M^+ +1).

Synthesis of 2-phenyl-3-(5-sulfanyl-1,3,4-oxadiazol-2-yl)-4*H*-4-chromenone (8): To a solution of compound **7** (0.01 mol) in ethyl alcohol (25 mL), potassium hydroxide (0.01 mol) and carbon disulfide (0.015 mol) and heated the reaction mixture under reflux with stirring for 12 hours, after the completion of the reaction, the solvent was removed by distillation *in vacuo*, the residual mass was poured over crushed ice and neutralized the alkaline solution with 10% hydrochloric acid. The precipitated crude product was filtered, washed with water, dried and recrystallized from ethanol to give pure compound **8**. IR (KBr) ν_{max}: 3082 (CH-Ar), 2536 (S-H), 1712 (C=O), 1642 (C=N), 1619 (C=C), 1193 (C-O-C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 5.89 (s, 1H, SH/NH), 7.50-7.60 (m, 6H, ArH), 7.95 (d, J = 8.8 Hz, 2H, ArH), 8.32 (d, J = 8.7 Hz, 1H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 119.5, 121.3, 123.7, 124.8, 125.6, 127.0, 129.2, 130.8, 133.3, 135.2, 154.8, 157.5, 165.0, 174.8, 175.4 ; MS: *m/z* 322 (M^+).

Synthesis of 3(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)-2-phenyl-4*H*-4-chromenone (9): To a warm solution of compound **8** (0.01 mol) in ethanol (25 mL), 80% hydrazine hydrate (0.015 mol) was added drop wise and heated under reflux for 6 hours. The solvent was distilled *in vacuo*, cooled and the solid separated were filtered, washed with cold ethanol and recrystallized from chloroform to give pure compound **9**. IR (KBr) ν_{max}: 3254 (NH_2), 3067 (CH-Ar), 2539 (S-H), 1712 (C=O), 1645 (C=N), 1617 (C=C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 5.30 (s, 2H, NH_2), 7.50-7.60 (m, 6H, ArH), 7.88 (d, J = 8.6 Hz, 2H, ArH), 8.27 (d, J = 8.8 Hz, 1H, ArH), 10.16 (s, 1H, SH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 118.8, 120.0, 123.8, 124.0, 124.9, 126.3, 127.0, 130.1, 133.7, 134.5, 139.6, 144.1, 153.8, 167.4, 172.9, ; MS: *m/z* 337 (M^+ +1).

General procedure for the synthesis of 2-phenyl-3-(6-aryl [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-4*H*-4-chromenone 10(a-j): To a solution of compound **9** (0.01 mol) in $POCl_3$ (10 mL), a solution of aromatic carboxylic acid (0.01 mol) in ethanol (15 mL) was added and the reaction mixture was heated under reflux for 12 hours under anhydrous conditions. The solvent was distilled *in vacuo*, the residual mass was poured over crushed ice and the excess $POCl_3$ was neutralized with 10% sodium bicarbonate solution. The solid thus separated was filtered, washed with 10% sodium bicarbonate solution and finally with water, dried and recrystallized from ethanol to give pure compounds **10**.

2-phenyl-3-(6-phenyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-4*H*-4-chromenone (10a): IR (KBr) ν_{max}: 3077 (CH-Ar), 1709 (C=O), 1641 (C=N), 1617 (C=C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.50-7.60 (m, 11H, ArH), 8.10 (d, J = 8.4 Hz, 2H, ArH), 8.39 (d, J = 8.6 Hz, 1H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 118.2, 119.5, 123.9, 125.8, 126.2, 126.9, 127.2, 128.0, 130.0, 131.9, 132.7, 133.0, 134.8, 135.0, 135.8, 146.4, 149.3, 154.9, 169.4, 172.2; MS: *m/z* 422 (M^+).

3-[6-(2-chlorophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]-2-phenyl-4*H*-4-chromenone (10b): IR (KBr) ν_{max}: 3073 (CH-Ar), 1708 (C=O), 1644 (C=N), 1615 (C=C), 691 (C-Cl) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.00-7.10 (m, 2H, ArH), 7.50-7.60 (m, 8H, ArH), 8.12 (d, J = 8.4 Hz, 2H, ArH), 8.36 (d, J = 8.6 Hz, 1H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 118.4, 118.4, 123.2, 125.0, 126.1, 126.8, 127.8, 128.0, 129.8, 131.2, 131.9, 132.7, 133.4, 134.2, 134.9, 135.7, 136.0, 145.8, 149.5, 155.2, 169.1, 172.7; MS: *m/z* 456 (M^+).

(3-[6-(4-chlorophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]-2-phenyl-4*H*-4-chromenone (10c): IR (KBr) ν_{max}: 3081 (CH-Ar), 1710 (C=O), 1642 (C=N), 1615 (C=C), 689 (C-Cl) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.50-7.60 (m, 10H, ArH), 8.14 (d, J = 8.4 Hz, 2H, ArH), 8.36 (d, J = 8.6 Hz, 1H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 118.6, 120.2, 122.7, 125.6, 126.4, 126.9, 127.5, 129.3, 130.2, 132.0, 133.5, 133.9, 134.6, 135.4, 136.1, 145.9, 149.9, 155.2, 169.1, 173.1; MS: *m/z* 456 (M^+).

3-[6-(4-methylphenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]-2-phenyl-4*H*-4-chromenone (10d): IR (KBr) ν_{max}: 3076 (CH-Ar), 2977 (CH-Ali), 1704 (C=O), 1643 (C=N), 1618 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.48 (s, 3H, CH_3), 7. 10 (d, J = 8.2 Hz, 2H, ArH), 7.50-7.60 (m, 8H, ArH), 8.09 (d, J = 8.4 Hz, 2H, ArH), 8.35 (d, J = 8.6 Hz, 1H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 28.1, 119.0, 119.9, 123.3, 124.9, 125.4, 126.2, 127.5, 130.2, 131.9, 132.7, 133.3, 133.9, 134.9, 135.6, 139.0, 147.2, 149.9, 155.3, 169.7, 171.3; MS: *m/z* 436 (M^+).

3-[6(4-hydroxyphenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]-2-phenyl-4*H*-4-chromenone (10e): IR (KBr) ν_{max}: 3398 (O-H), 3081 (CH-Ar), 1711 (C=O), 1647 (C=N), 1619 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.21 (s, 1H, OH), 7.00-7.05 (4H, m, ArH), 7.50-7.60 (m, 6H, ArH), 8.12 (d, J = 8.4 Hz, 2H, ArH), 8.37 (d, J = 8.6 Hz, 1H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 115.3, 118.9, 120.6, 123.2, 124.8, 125.4, 126.2, 127.7, 128.2, 133.2, 133.9, 134.1, 134.9, 135.4, 145.7, 149.4, 153.4, 159.2, 170.1, 173.5; MS: *m/z* 439 (M^++1).

(3-[6(4-methoxyphenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]-2-phenyl-4*H*-4-chromenone (10f): IR (KBr) ν_{max}: 3079 (CH-Ar), 2988 (CH-Ali), 1704 (C=O), 1647 (C=N), 1621 (C=C), 1077 (C-O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.93 (s, 3H, OCH_3), 6.81 (d, J = 8.5 Hz, 2H, ArH), 7.50-7.60 (m, 8H, ArH), 8.14 (d, J = 8.4 Hz, 2H, ArH), 8.41 (d, J = 8.6 Hz, 1H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 56.9, 116.3, 119.2, 120.1, 123.4, 124.6, 125.4, 126.2, 127.8, 129.7, 131.9, 132.0, 133.4, 134.7, 135.1, 146.9, 150.1, 154.3, 158.0, 169.0, 171.7; MS: *m/z* 452 (M^+).

3-[6-(3-nitrophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]-2-phenyl-4*H*-4-chromenone (10g): IR (KBr) ν_{max}: 3082 (CH-Ar), 1708 (C=O), 1649 (C=N), 1618 (C=C), 1572, 1370 (NO_2) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.50-7.60 (m, 6H, ArH), 8.00-8.10 (m, 6H, ArH), 8.35 (d, J = 8.6 Hz, 1H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 118.3, 119.1, 123.0, 124.7, 125.0, 125.5, 126.0, 126.3, 127.6, 131.2, 132.4, 133.5, 134.4, 135.9, 136.0, 138.7, 146.2, 148.9, 149.1, 154.2, 169.7, 172.8; MS: *m/z* 467 (M^+).

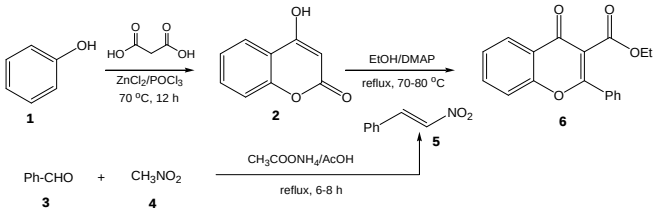
3-[6-(4-nitrophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]-2-phenyl-4*H*-4-chromenone (10h): IR (KBr) ν_{max}: 3091 (CH-Ar), 1711 (C=O), 1645 (C=N), 1616 (C=C), 1572, 1369 (NO_2) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.50-7.60 (m, 6H, ArH), 8.00-8.10 (m, 6H, ArH), 8.33 (d, J = 8.6 Hz, 1H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 119.1, 120.4, 123.7, 124.9, 125.5, 125.9, 126.2, 127.4, 128.2, 133.2, 133.9, 134.1, 135.8, 139.9, 145.7, 147.7, 148.2, 153.5, 169.9, 172.0; MS: *m/z* 467 (M^+).

3-(6-benzyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-2-phenyl-4*H*-4-chromenone (10i): IR (KBr) ν_{max}: 3081 (CH-Ar), 2991 (CH-Ali), 1712 (C=O), 1649 (C=N), 1615 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.13 (s, 2H, CH_2), 6.72 (d, J = 8.1 Hz, 2H, ArH), 7.50-7.60 (m, 9H, ArH), 8.13 (d, J = 8.4 Hz, 2H, ArH), 8.36 (d, J = 8.6 Hz, 1H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 42.1, 118.7, 120.2, 123.4, 124.8, 125.6, 126.8, 127.1, 127.5, 128.8, 130.1, 132.9, 133.2, 134.1, 135.9, 138.2, 146.5, 150.4, 153.2, 169.7, 172.1; MS: *m/z* 436 (M^+).

3-[6-(4-chlorobenzyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]-2-phenyl-4*H*-4-chromenone (10j): IR (KBr) ν_{max}: 3071 (CH-Ar), 2992 (CH-Ali), 1709 (C=O), 1642 (C=N), 1616 (C=C), 691 (C-Cl) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.13 (s, 2H, CH_2), 6.82 (d, J = 82. Hz, 2H, ArH), 7.50-7.60 (m, 8H, ArH), 8.13 (d, J = 8.4 Hz, 2H, ArH), 8.31 (d, J = 8.6 Hz, 1H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 42.7, 118.9, 120.1, 123.2, 125.7, 125.9, 127.2, 128.0, 128.9, 132.5, 133.2, 133.9, 134.0, 134.9, 135.5, 136.8, 146.2, 149.1, 155.7, 169.0, 172.7; MS: *m/z* 470 (M^+).

RESULTS AND DISCUSSION

The compound 2 was prepared according to the procedure reported in the literature (Naveen, 2006), by cyclo-condensation of phenol 1 with malonic acid in the presence of phosphorous oxychloride and anhydrous zinc chloride under reflux on a water bath at 70 ^{o}C for 12 h, gave the 4-hydroxy-2*H*-2-chromenone 2 as light yellow powder. The compound 5 also prepared by the literature procedure (Shafi, 2016), by condensation of benzaldehyde 3 with nitromethane 4 in the presence of ammonium acetate in acetic acid at reflux temperature for 6-8 h, resulted the nitrostyrene 5(a-j). Further, compound 2 was reacted with compound 5 in the presence of dimethylaminopyridine (DMAP) (Bhattacharjee, 2016) in ethyl alcohol at reflux temperature to afford the ethyl 4-oxo-2-phenyl-4*H*-3-chromenecarboxylate 6 (Scheme 1). The structure of compound 6 was confirmed by is EI mass, IR, ^{1}H NMR and ^{13}C NMR spectral data.

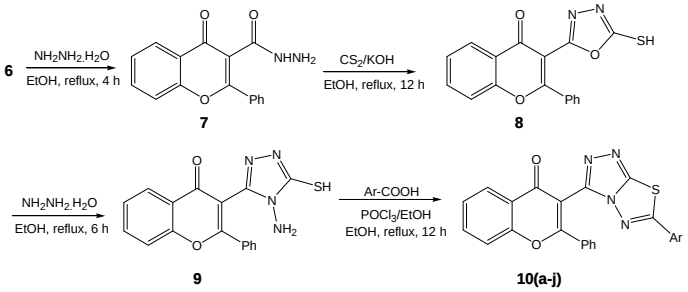


Scheme 1. Schematic route for the synthesis of compounds 6

The IR spectrum of compound 6, the ester carbonyl (C=O) and (C-O) absorption band appeared at 1734, 1278 cm^{-1} and the carbonyl (C=O) of flavone ring observed at 1678 cm^{-1}. Its proton NMR spectrum, the methyl and methylene protons of ester appeared at δ 1.32 as triplet and δ 4.56 as quartet, the aryl proton signals appeared as multiplet at δ 7.45-7.55, and two doublets at δ 7.92, 8.22 ppm. Its ^{13}C NMR spectrum, the signals of carbons of flavone ring appeared at δ 170.8 (C2), 110.9 (C3), 174.4 (C4), 123.4 (C5) and 156.6 (C6).

The compound 6 was reacted with hydrazine hydrate in ethanol at reflux for 4 h, to get the 4-oxo-2-phenyl-4*H*-3-chromenecarbohydrazide 7, which on cyclo-condensation of with carbondisulfide in the presence of potassium hydroxide in ethanol under reflux for 12 hours, followed by acidification, afforded 2-phenyl-3-(5-sulfanyl-1,3,4-oxadiazol-2-yl)-4*H*-4-chromenone 8 in good yield. The compound 8, when reacted with hydrazine hydrate in ethanol at reflux temperature for 6 hours afforded the 3-(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)-2-phenyl-4*H*-4-chromenone 9, which further reacted with corresponding aromatic carboxylic acids in the presence of $POCl_3$ in ethanol at reflux temperature for 12 hours, afforded the title compounds, synthesis of 2-phenyl-3-(6-aryl[1,2,4] triazolo[3,4-*b*][1,3,4] thiadiazol-3-yl)-4*H*-4-chromenone 10(a-j) (Scheme 2) in 48-66% yields. The structures of all the newly synthesized compounds were confirmed by their EI mass, IR, ^{1}H NMR and ^{13}C NMR spectral data. The IR spectrum of 7

displayed stretching bands for amine (N-H) group at 3412, carbonyl (C=O) group of flavone at 1712, carbonyl (C=O) group of hydrazide at 1682 and amide (C-N) group at 1369 cm^{-1}; Its ^1H NMR spectrum, displayed a singlet signal at δ 5.52 and δ 8.92 which were accounted for NH and NH_2 respectively, the aryl proton signals appeared as multiplets at δ 7.45-7.55, 7.70-7.80, and a doublet at δ 8.21 with the coupling constant J = 8.6 Hz. Its ^{13}C NMR spectrum, showed signals at δ 166.8 (C2), 110.4 (C3), 173.8 (C4), 123.2 (C5) and 119.0 (C6) carbons of flavones ring. Its mass spectrum displayed a molecular ion peak at m/z: 280 which confirmed its molecular weight.



10: Ar = a) phenyl; b) 2-chlorophenyl; c) 4-chlorophenyl; d) 4-methylphenyl; e) 4-hydroxyphenyl; f) 4-methoxyphenyl; g) 3-nitrophenyl; h) 4-nitrophenyl; i) benzyl; j) 4-chlorobenzyl.

Scheme 2. Schematic route for the synthesis of compounds 10(a-j)

The IR spectrum of **8** displayed stretching bands for S-H group at 2536, carbonyl (C=O) group of flavone at 1712, the absence of hydrazide carbonyl stretching and presence of absorption bands of C=N, C-O-C of oxadiazole ring at 1643, 1193 cm^{-1} confirmed the formation of oxadiazole ring involving hydrazide group. Its ^1H NMR spectrum, displayed a singlet signal at δ 5.89 which is accounted for SH/NH proton, the aryl proton signals appeared as multiplets at δ 7.50-7.60 and doublets at δ 7.95 and 8.92 ppm. Its ^{13}C NMR spectrum, showed signals at δ 165.0 (C2) and 157.5 (C5) carbons of oxadiazole ring. Its mass spectrum displayed a molecular ion peak at m/z: 322 which confirmed its molecular weight. The IR spectrum of **9** displayed stretching bands at 3254 and 2539 cm^{-1} due to N-H and S-H groups, the carbonyl (C=O) group of flavone at 1712, C=N of oxadiazole ring at 1645 cm^{-1}. Its ^1H NMR spectrum, displayed a singlet signal at δ 5.30 and 1016 ppm which were accounted for NH_2 and SH protons respectively, the aryl proton signals appeared as multiplets at δ 7.50-7.60 and doublets at δ 7.88 and 8.27 ppm. Its ^{13}C NMR spectrum showed signals at δ 144.1 (C2) and 139.6 (C5) carbons of oxadiazole ring. Its mass spectrum displayed a molecular ion peak at m/z: 337 which confirmed its molecular weight.

The IR spectrum of 10a, the carbonyl (C=O), alkene (C=C) groups of flavones and C=N of the oxadiazole ring showed abosoption band at 1709, 1617 and 1641 cm^{-1}, further, the absence of -NH_2 and -SH stretching vibrations provided the evidence of ring closure, involving -NH_2 and -SH groups. Similarly, the absence of signals for the -SH and -NH_2 protons in the ^1H NMR spectra followed by the presence of aromatic protons as multiplet at δ 7.50-7.60 7.60, doublets at δ 8.10 and 8.30 ppm well supported the structures. Its ^{13}C NMR spectra, the prominent signals corresponding to the carbons of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole ring for all the compounds, observed nearly at δ 149.3, 146.4 and 132.7 ppm are proof of further evidence of its structures. Its mass spectrum displayed a molecular ion peak at m/z: 422 which confirmed its molecular weight. All the newly synthesized compounds exhibited satisfactory spectral data consistent with their molecular structures.

ANTIBACTERIAL ACTIVITY

All newly synthesized compound **10(a-j)** were screened for their *in vitro* antibacterial activities against bacterial strains of *Bacillus subtilis*, *Micrococcus luteus*, *Proteus mirabilis, and Escherichia coli* using the disc diffusion method (Seely, 1975). The zone of inhibition (mm) at 500 μg/mL of the test compound were determined and compared with the standard antibacterial drug ciprofloxacin, the results have been reported in Table 1.

Table 1. Antibacterial activity of compounds 10(a-j)

Compound	Zone of inhibition (mm) at 500 μg/mL			
	B. subtilis	*M. luteus*	*P. mirabilis*	*E. coli*
10a	10	13	—	—
10b	19	17	21	16
10c	22	18	24	18
10d	15	14	9	11
10e	18	20	12	9
10f	19	18	21	17
10g	13	10	—	—
10h	11	9	7	—
10i	12	10	7	5
10j	16	13	11	9
Ciprofloxacin	23	20	25	20

Antibacterial evaluation of compounds 5(a-j) indicates, that these compounds showed considerable inhibition towards all the tested bacteria. Amongst them, compounds containing 2-chloroyphenyl (8d), 4-chlorophenyl (8e) and 4-nitrophenyl (8h) groups on piperazine ring showed considerable activity against tested bacterial strains. Compound, 8d showed better activity against *S. aureus* and *M. Luteus*, compound (8e) showed better activity against Gram-positive and Gram-negative bacteria (except *E. coli)*. Similarly, compounds containing 4-nitrophenyl (8h) was active against all the tested strains *except S. Aureus*. The other compounds also exhibited considerable antibacterial activities.

Conclusion

In conclusion, a new series of 2-phenyl-3-(6-aryl[1,2,4]triazol [3,4-*b*][1,3,4]thiadiazol-3-yl)-4*H*-4-chromenone 10(a-j) has been synthesized and screened for their *in vitro* antibacterial activitiy. Most of the new compounds showed considerable activity, among them compounds containing 2-chloropheny (10b), 4-chlorophenyl (10c), 4-methoxyphenyl (10f) and 4-hydroxyphenyl (10e) showed significant activity.

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